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IMPROVED METHOD FOR THE PREPARATION OF VITAMINE-ACTIVATED FULLER'S EARTH.

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Since the discovery several years ago¹ that certain varieties of fuller's earth, particularly the variety from Surrey, England, exert a remarkable adsorptive power for the antineuritic vitamine, this reagent has been exclusively used as the first step in my attempts to isolate vitamine from brewer's yeast. For this purpose the fresh yeast was allowed to autolyze, and the resulting product was filtered. Fuller's earth was then added to the clear liquid and, after thorough agitation, the solid was removed by filtration and was washed and dried. The "activated fuller's earth" thus prepared, on account of its relative uniformity and exceptional stability, was believed to be the most favorable raw material for experiments on the isolation of the antineuritic vitamine.

The most unsatisfactory step in the process of preparing "activated solid" is the extremely slow and wasteful filtration of the autolyzed yeast. Numerous experiments were early made for the purpose of eliminating this very troublesome filtration, but improvements worthy of recommendation were not developed. Recently, however, by making use of an observation of Osborne and Wakeman,² a substantial improvement in the method has been made and is now brought to the attention of persons interested in the more precise study of the chemical and physiological properties of the antineuritic vitamine.

It was demonstrated by Osborne and Wakeman that when fresh yeast is added to boiling water, acidified with 0.01 per cent acetic acid, the yeast cells are disrupted, the protein is coagulated, and the vitamine is set free to enter the aqueous solution. This latter can readily be separated from the coagulated protein and insoluble material and is a very much more satisfactory solution for the adsorption of vitamine by fuller's earth than the very complex filtrate from autolyzed yeast.

The advantages resulting from this modification are that the manipulations are greatly shortened and simplified and, what is of

¹ Seidell: Public Health Reports, 31, 384 (1916).

² Osborne and Wakeman: J. Biol. Chem., 40, 383 (1919).

greater importance, a much better final product is obtained. The basis of the latter claim is that at least one of the interfering substances simultaneously adsorbed with the vitamine is almost, if not entirely, eliminated. This compound is adenine, which is a product of the autolysis and is present in the yeast filtrate and subsequently adsorbed by the fuller's earth. It is, however, not formed to an appreciable extent during the rapid heating of the fresh yeast according to the modified method, and consequently does not become an impurity in the final "activated solid."

The improved method for preparing vitamine-activated fuller's earth on a moderately large scale has been very satisfactorily carried out as follows:

Fresh bottom yeast, as obtained from the brewery, in quantities of 50 or more liters, is diluted with about an equal volume of tap water and heated, while being stirred, in a steam-jacketed kettle until the temperature reaches approximately 90° C. It has been found that if this temperature is exceeded, the mixture is apt to foam excessively and overflow the kettle. After about 5 minutes at 90°, the mixture is cooled to 50° or less, and the coagulated protein is removed by filtration.

The protein can be very effectively and expeditiously removed by means of a large Sharples centrifuge. The protein remaining in the bowl of this machine is of cheese-like consistency and retains a relatively small proportion of the aqueous vitamine solution. This latter is a clear dark-brown liquid. English fuller's earth is added to it in the proportion of 30 grams per liter and the mixture is actively stirred for one-half hour or longer. It is then subjected to filtration or the solid may be removed more conveniently by means of the Sharples centrifuge. The "activated solid" is finally washed with several changes of water and alcohol and then thoroughly dried to prevent subsequent growth of molds, which are very readily nourished by the minute amounts of organic material from the yeast solution, persistently retained by the "activated solid."

Samples of "activated solid" prepared by the above process were found to contain approximately 1.5 per cent of nitrogen instead of slightly more than the 2 per cent usually present in samples prepared by the original method from autolyzed yeast filtrate. The content of antineuritic vitamine, as estimated by feeding experiments on pigeons, was found to be about twice as great as that of the product made by the original method. Complete protection of pigeons against loss in weight on a diet of polished rice, or restoration of such lost weight, was afforded in all cases by doses of 0.1 gram on alternate days. Doses of 0.05 gram prevented all but a very slight diminution in weight for many weeks.

From these tests it is evident that the protective dose for pigeons of 300 grams weight is under 0.1 gram; whereas with samples of "acti-

vated solid," prepared by the original method, adequate protection usually required doses between 0.1 and 0.2 gram. This, taken in connection with the lower nitrogen content and absence of adenine is ample evidence of the greatly improved character of the "activated solid" prepared by the new method.

HAY-FEVER PLANTS OF CALIFORNIA.

By HARVEY MONROE HALL, B. S., M. S., Ph. D., formerly head of the Department of Botany, University of California; Member California Academy of Sciences.

1. PURPOSE OF THE PAPER.

The purpose of the present paper is to assist the members of the medical profession in determining the specific causes of hay fever in their respective districts. The lists here presented are intended to include all of the more important hay-fever plants of California. It is hoped that with the aid of the notes accompanying the catalogue the specialist can inform himself as to the plants that are the most frequent causes in his particular locality. The pollen or pollen extracts of these species may then be obtained and used in skin-reaction tests. In this manner the exact botanical species responsible for the hay fever of the individual may be determined, assuming that the symptoms are not due to some cause other than pollen. The final procedure is the desensitization of the patient through the use of the extracts to which positive reactions have been obtained, this treatment preferably to precede the period of pollination.

The lists here assembled and the principles used in the selection of species are the result of field studies in all parts of the State, extending over a long period of time. In recent years special attention has been given to plants as causes of hay fever, and there has been frequent opportunity for consultation with practicing specialists. The author is especially indebted to Dr. Grant Selfridge of San Francisco, who first called his attention to the subject, and the results of whose studies have been freely available throughout this period. Reports on the results of skin-reaction tests have been submitted also by the following: Dr. William P. Scheppegegrell, president of the American Society for the Prevention of Hay Fever; Dr. Charles T. Chamberlain, Portland, Oreg.; Dr. George Piness, Los Angeles, Calif.; Dr. Albert H. Rowe, Oakland, Calif.; and Dr. Samuel H. Watson, Tucson, Ariz. Their findings have been incorporated into the present paper in so far as they apply to Californian plants. The author's thanks are due to these specialists and to many others, both in California and in the Eastern States, for helpful data and advice.